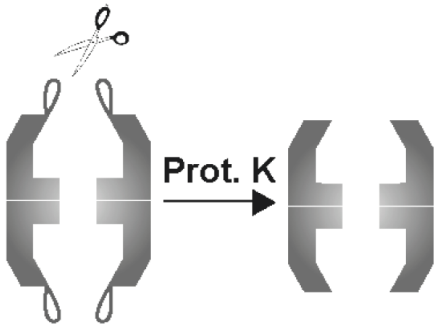
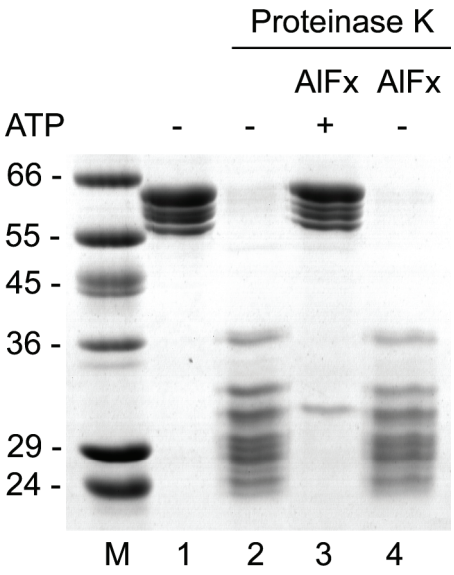
SUPPLEMENTARY FIGURE LEGENDS

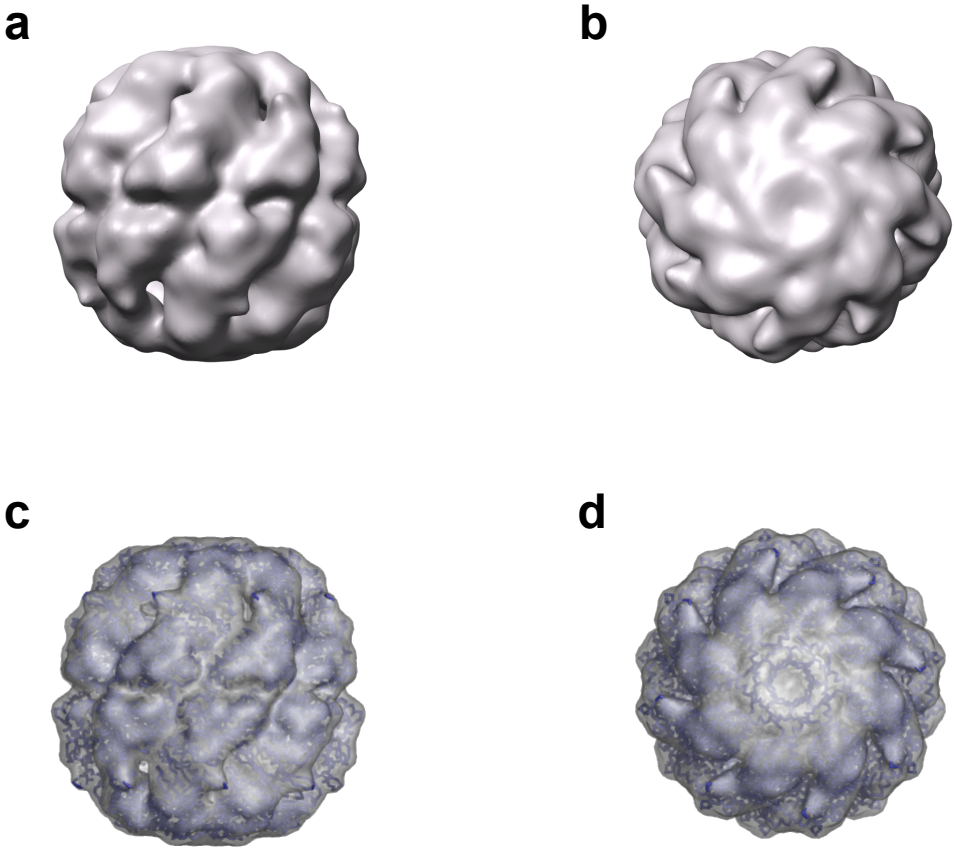
Booth et al

Mechanism of lid closure in the eukaryotic chaperonin TRiC/CCT

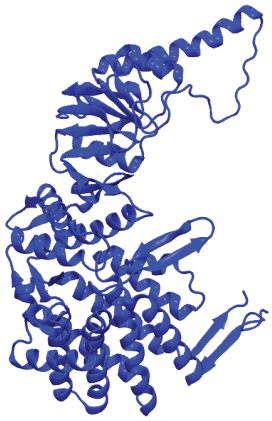

Prot. K


Proteinase K
AlFx AlFx
ATP - - + -
66 -
55 -
45 -
36 -
29 -
24 -
M 1 2 3 4

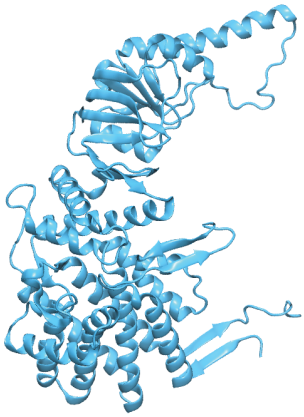
Supplementary Figure 1 Incubation with ATP and AlF_x induces symmetrical lid closure of TRiC. TRiC-nucleotide complexes formed by preincubation were subjected to proteolytic treatment, analyzed by SDS-PAGE, and stained with Coomassie blue. Nucleotide-free TRiC (lane 2) is proteolyzed at the lid-forming apical protrusions of its subunits due to its open lids. In the presence of both ATP and aluminum fluoride (lane 3), the TRiC complex is completely protected from proteolytic cleavage, indicating that both lids are in a closed conformation. Aluminum fluoride alone (lane 4) did not fully protect the structure from cleavage. Lane M: molecular weight standards.


a
b
c
d

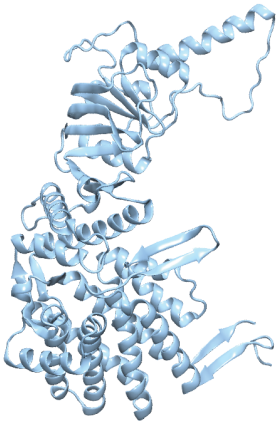
Supplementary Figure 2 The closed TRiC state resembles the thermosome crystal structure. Blurring of the thermosome crystal structure (blue trace, **a**, **b**) to a resolution equivalent to that of the TRiC closed state density map (gray density) reveals a marked resemblance between the two structures (compare thermosome density map, **c**, **d** with TRiC density map, **e**, **f**). The visual similarity is confirmed quantitatively by a similarity score of 0.945 when the thermosome crystal structure is docked into the TRiC closed state cryo-EM density (**c**, **d**) using the *foldhunter* program[57]. The similarity score was found to be 3.36 standard deviations above the mean similarity score. The high degree of structural similarity supports that it was reasonable to apply the 8-fold symmetry during the image reconstruction process at the current resolution. Furthermore, this comparison shows that TRiC in the closed state adopts a conformation that is almost identical to that determined for the thermosome subunits.



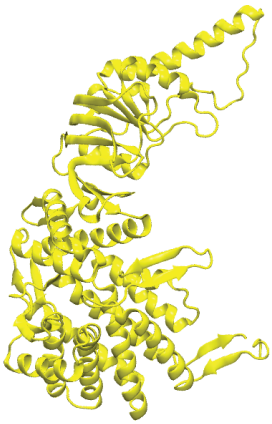
γ subunit



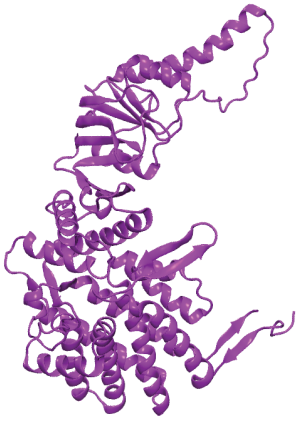
β subunit



ζ subunit

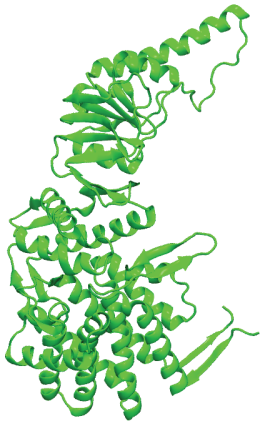


δ subunit



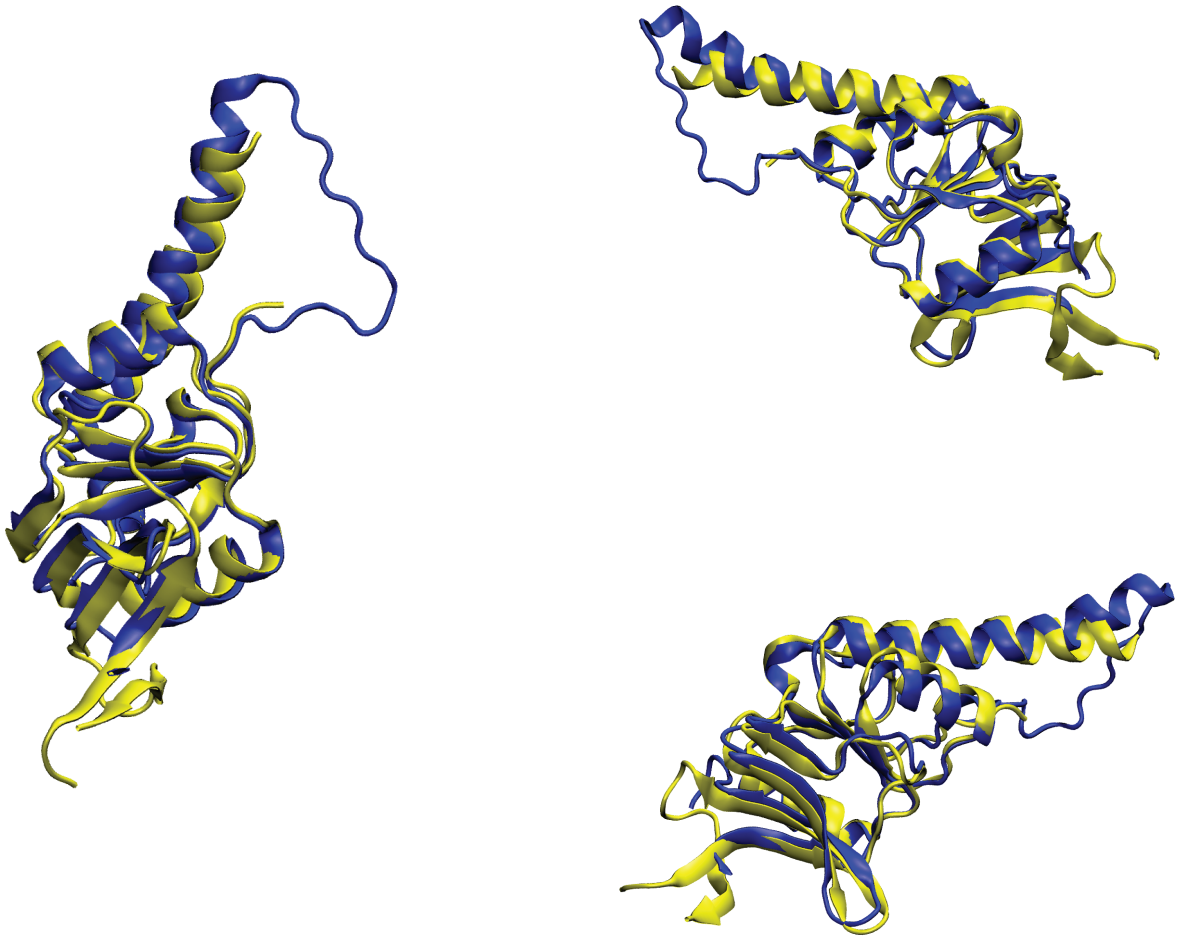
θ subunit

ε subunit



η subunit

Supplementary Figure 3 Comparative models for individual TRiC subunits. The models for seven subunits of the TRiC closed state are shown here; the eighth subunit, α, is shown in Figure 1B. The models were derived as described in the text. Our comparative protein structure modeling approach is validated by the excellent match between the model for subunit γ and the experimentally determined crystal structure of the apical domain of this subunit (Supplementary Figure 4).



Supplementary Figure 4 Overlay of the comparative model for subunit γ and the crystal structure of the apical domain of the mouse γ subunit. The mouse CCT γ apical domain structure is shown in yellow (PDB ID: 1GML), the homology model in blue. The RMSD between Cα atoms of the corresponding residues is 1.5 Å. The excellent agreement between the γ subunit model and the X-ray structure of its mouse homologue validate our structural homology models.

SUPPLEMENTARY MOVIES

Supplementary Movie 1 Monomer transition from open to closed conformation.

Supplementary Movie 2 Octamer transition from open to closed conformation.

Supplementary Movie 3 Top view of the lowest frequency non-degenerate mode (mode 1) from normal mode analysis (NMA). Only Cα atoms are illustrated in space filling style.

Supplementary Movie 4 Side view of the lowest frequency non-degenerate mode (mode 1) from NMA. Same illustration style as in Supplementary Movie 3.